

Figure 1

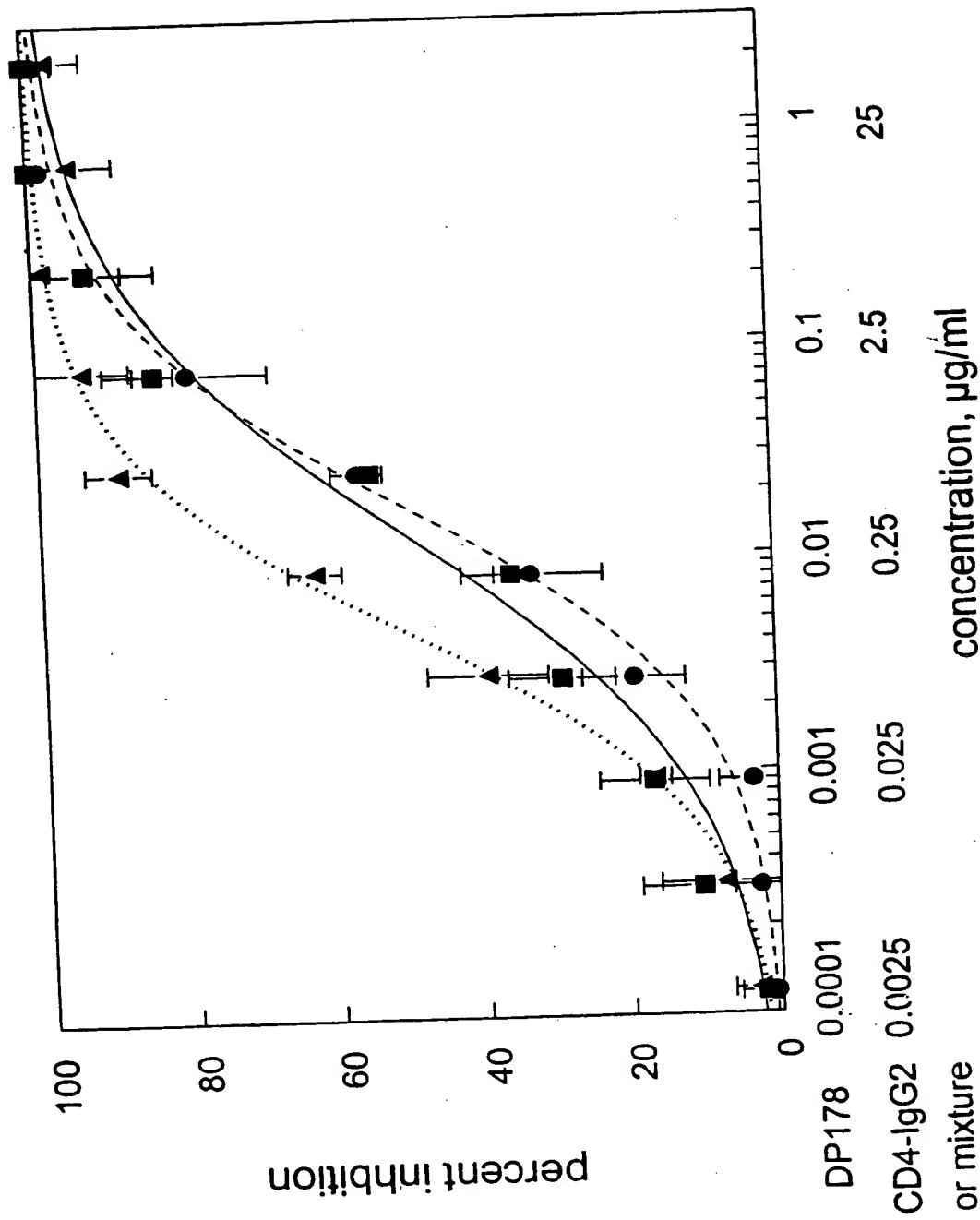


Figure 2

Percent Inhibition	Combination Index			
	CD4-IgG2:T-20 Mass Ratio			
	25:1 (low)	25:1 (high)	5:1	1:1
95	0.32	0.20	0.22	0.50
90	0.38	0.25	0.27	0.55
85	0.43	0.29	0.30	0.59
80	0.47	0.33	0.34	0.62
75	0.51	0.36	0.37	0.65
70	0.54	0.39	0.40	0.67
65	0.58	0.42	0.43	0.70
60	0.61	0.45	0.45	0.73
55	0.65	0.48	0.49	0.75
50	0.69	0.51	0.52	0.78

Figure 3
 TABLE 3

Percent Inhibition	T-20			CD4-IgG2		
	Concentration, $\mu\text{g/ml}$		Dose Reduction	Concentration, $\mu\text{g/ml}$		Dose Reduction
	Alone	Combination		Alone	Combination	
99	1.1	0.17	6.6	130	4.3	29
95	0.21	0.044	4.9	19	1.10	17
90	0.10	0.024	4.2	7.8	0.59	13
70	0.025	0.0076	3.3	1.6	0.19	8.4
50	0.011	0.0039	2.8	0.60	0.095	6.3

Figure 4A

		PRO 542				PA12				T-20			
Percent Inhibition	Combination Index	Concentration, nM		Dose Reduction		Concentration, nM		Dose Reduction		Concentration, nM		Dose Reduction	
		Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix
95	0.41	10	2.1	4.8	2.1	730	2.8	260	2.8	94	19	4.9	4.9
90	0.45	7.0	1.6	4.4	1.6	320	2.1	150	2.1	63	14	4.5	4.5
70	0.47	4.1	0.92	4.5	0.92	72	1.2	60	1.2	30	8.1	3.7	3.7
50	0.48	3.1	0.66	4.7	0.66	28	0.87	32	0.87	19	5.8	3.3	3.3

PRO 542, PA12 and T-20 were used in an approximate 1:1:10 molar concentration ratio.

Figure 4B

		PRO 542				PRO 140				T-20			
Percent Inhibition	Combination Index	Concentration, nM		Dose Reduction		Concentration, nM		Dose Reduction		Concentration, nM		Dose Reduction	
		Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix
95	0.40	8.5	1.9	4.5	1.0	19	1.0	19	17	140	17	8.2	
90	0.39	7.1	1.5	4.7	0.77	13	0.77	17	13	100	13	7.7	
70	0.37	5.3	0.87	6.1	0.46	7.2	0.46	16	7.7	57	7.7	7.4	
50	0.35	4.6	0.63	7.3	0.34	4.9	0.34	14	5.6	40	5.6	7.1	

PRO 542, PRO 140 and T-20 were used in an approximate 2:1:20 molar concentration ratio.

Figure 4C 72324600

Percent Inhibition	Combination Index	PRO 542				PRO 140				T-20			
		Concentration, nM		Dose		Concentration, nM		Dose		Concentration, nM		Dose	
		Alone	Mix	Reduction	Alone	Alone	Mix	Reduction	Alone	Alone	Mix	Reduction	Alone
95	0.24	61	2.5	24	11.9	0.72	17	156	22	7.1			
90	0.22	32	1.4	23	8.4	0.40	21	96	13	7.4			
70	0.19	9.8	0.50	20	4.5	0.14	32	40	4.5	8.9			
50	0.18	4.7	0.26	18	3.0	0.074	41	23	2.3	10			

PRO 542, PRO 140 and T-20 were used in an approximate 4:1:30 molar concentration ratio.

Figure 4D: IC_{50} values

Percent Inhibition	Combination Index	PRO 140		T-20	
		Concentration, nM		Concentration, nM	
		Alone	Mix	Alone	Mix
95	0.56	12	1.8	6.7	156
90	0.55	8.4	1.1	7.4	96
70	0.55	4.5	0.51	8.8	40
50	0.56	3.0	0.31	9.9	23
					10
					2.4

PRO 140 and T-20 were used in an approximate 1:30 molar concentration ratio.

Figure 5

Triple Combination Synergistically Blocks HIV-1 Entry (I)

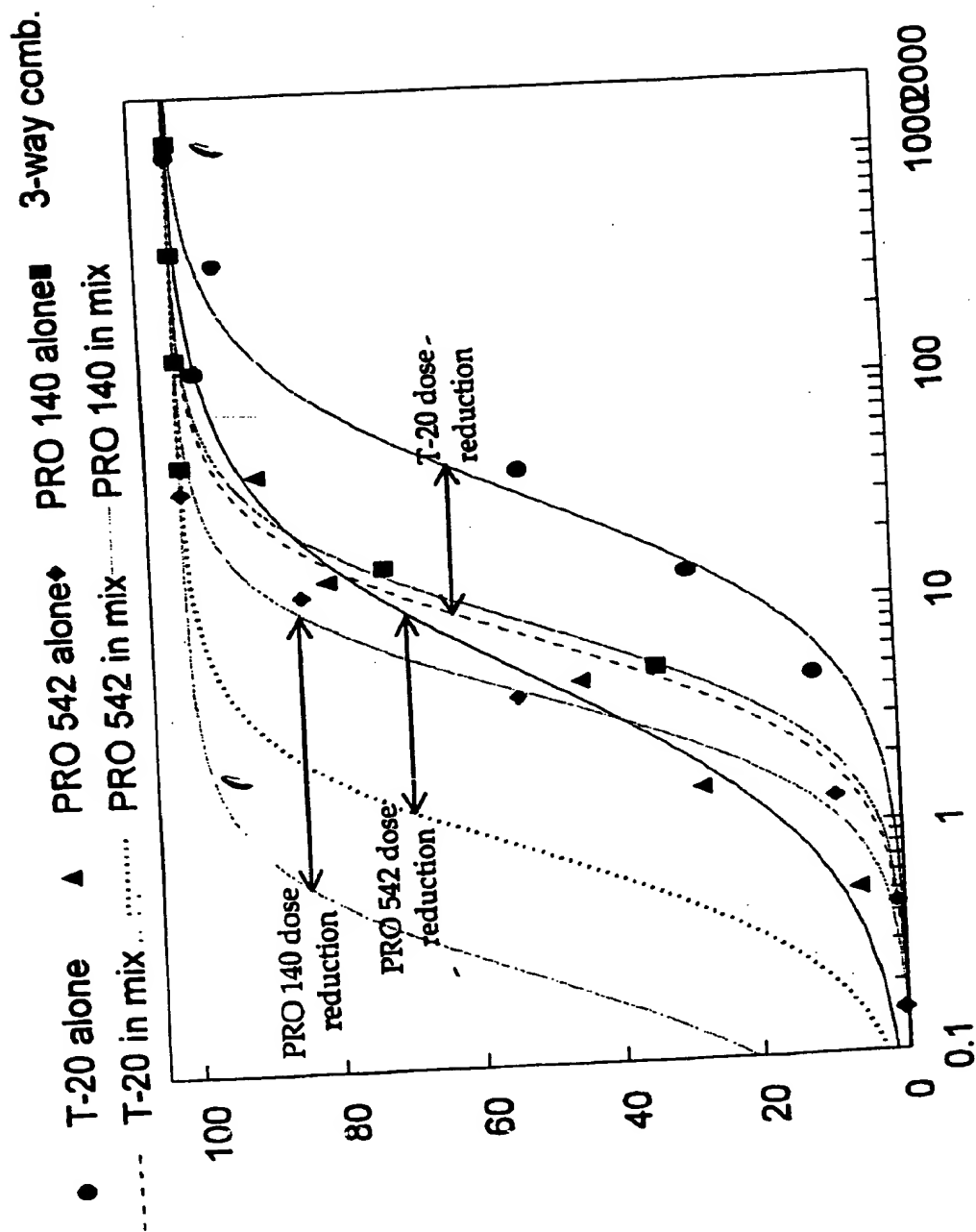


Figure 6 "The effect of DP178 on CD4-IgG2 mixture"

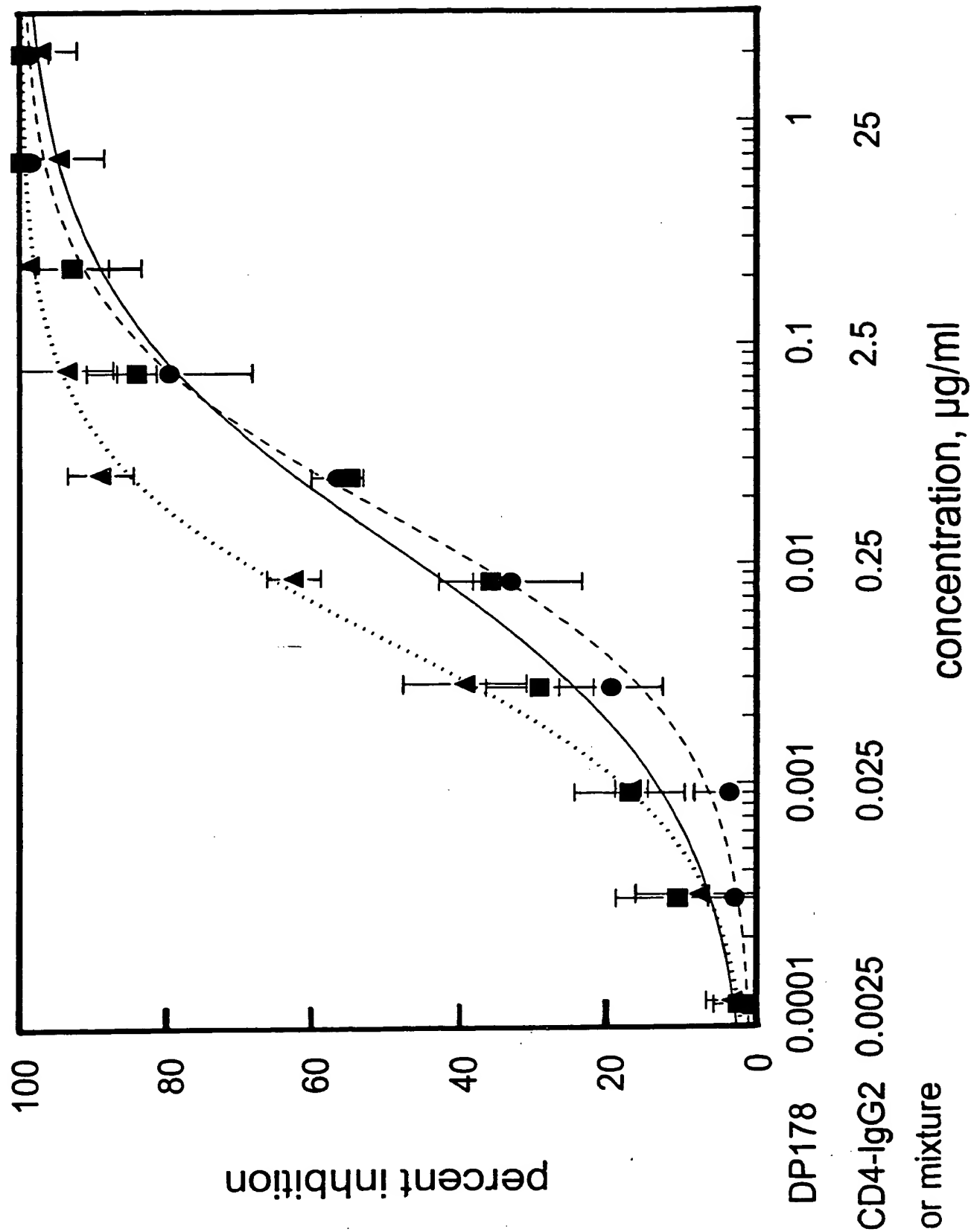


Figure 7
CD4-IgG2:T-20

Combination Index				
CD4-IgG2:T-20 Mass Ratio				
Percent Inhibition	25:1 (low)	25:1 (high)	5:1	1:1
95	0.32	0.20	0.22	0.50
90	0.38	0.25	0.27	0.55
85	0.43	0.29	0.30	0.59
80	0.47	0.33	0.34	0.62
75	0.51	0.36	0.37	0.65
70	0.54	0.39	0.40	0.67
65	0.58	0.42	0.43	0.70
60	0.61	0.45	0.45	0.73
55	0.65	0.48	0.49	0.75
50	0.69	0.51	0.52	0.78

Figure 8

Percent Inhibition	T-20				CD4-IgG2			
	Concentration, $\mu\text{g/ml}$		Dose Reduction		Concentration, $\mu\text{g/ml}$		Dose Reduction	
	Alone	Combination	Alone	Combination	Alone	Combination	Alone	Combination
99	1.1	0.17	6.6		130	4.3		29
95	0.21	0.044	4.9		19	1.10		17
90	0.10	0.024	4.2		7.8	0.59		13
70	0.025	0.0076	3.3		1.6	0.19		8.4
50	0.011	0.0039	2.8		0.60	0.095		6.3

Figure 9

Assay (virus)	PRO 542:T-20 Molar Ratio	Percent Inhibition	Combination Index	PRO 542				T-20			
				Concentration, nM		Dose Reduction	Concentration, nM	Concentration, nM		Dose Reduction	
				Alone	Mix		Alone	Alone	Mix		
Virus-cell fusion (JR-FL)	1:2	95 90 70 50	0.14 0.18 0.29 0.39	30 12 2.5 0.92	2.8 1.5 0.44 0.21	11 8.0 5.7 4.4	120 45 8.0 2.7	5.1 2.6 0.78 0.37	24 17 10 7.3		
Virus-cell fusion (DH123)	1:2	95 90 70 50	0.36 0.45 0.76 1.1	65 20 2.4 0.64	11 5.0 1.2 0.49	5.9 4.0 2.0 1.3	123 54 12 4.8	20 8.9 2.1 0.87	6.2 6.1 5.7 5.5		
Cell-cell fusion (JR-FL)	1:2	95 90 70 50	0.36 0.43 0.61 0.76	35 14 2.9 1.0	6.3 3.2 0.94 0.43	5.6 4.4 3.1 2.3	73 34 8.5 3.6	11 5.8 1.7 0.78	6.6 5.9 5.0 4.6		
Cell-cell fusion (JR-FL)	1:10	95 90 70 50	0.27 0.28 0.31 0.34	28 11 2.3 0.84	1.4 0.55 0.11 0.039	20 20 21 17	58 22 3.8 1.3	12 4.9 0.97 0.35	4.8 4.5 3.9 3.7		
Cell-cell fusion (JR-FL)	1:50	95 90 70 50	0.33 0.34 0.36 0.38	47 15 1.8 0.49	0.84 0.30 0.045 0.014	56 50 40 35	120 42 6.1 1.8	37 13 2.0 0.61	3.2 3.2 3.0 3.0		

Figure 10

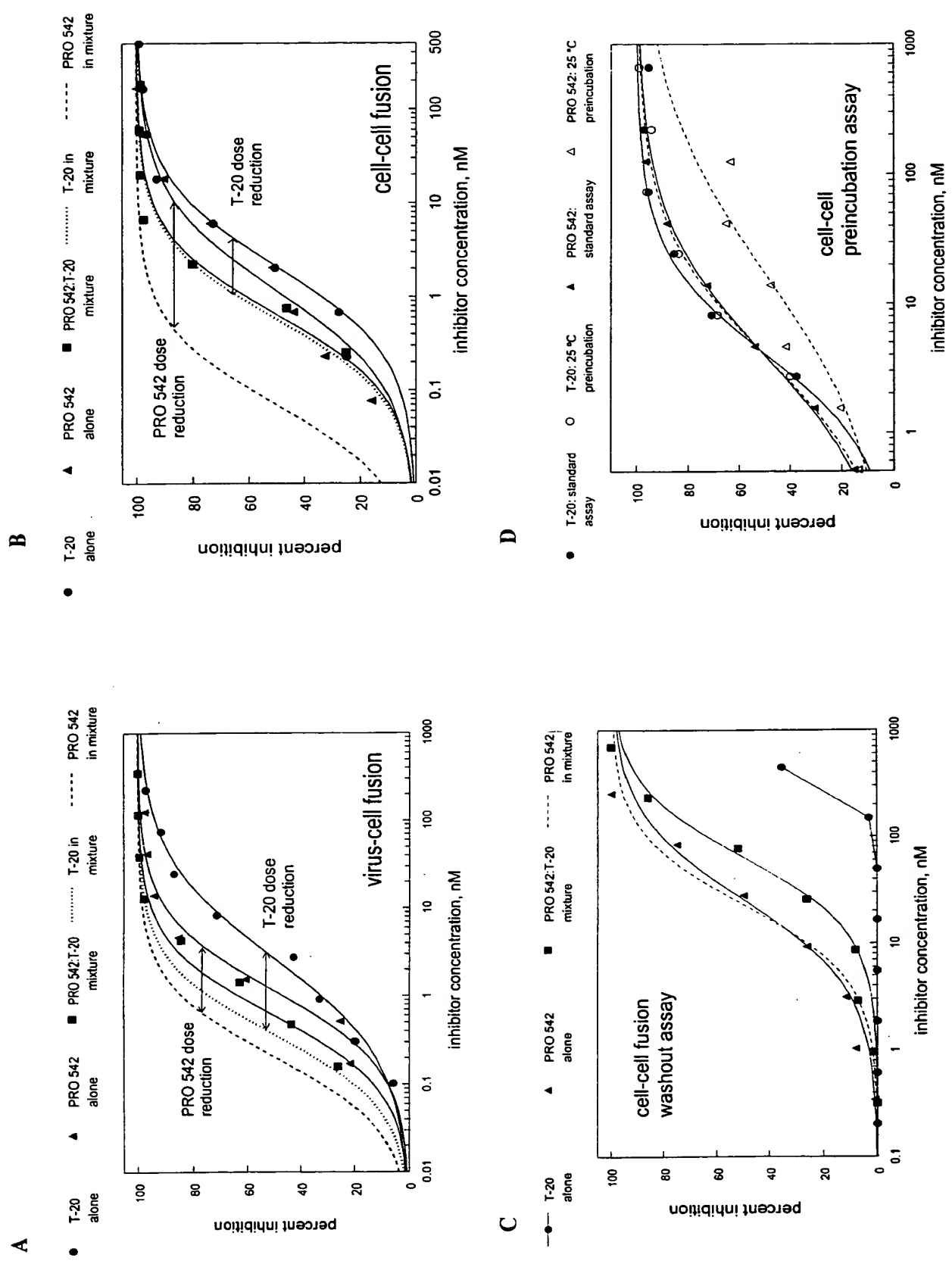


Figure 11 The 660

HIV-1 Entry Involves at Least Three Steps that Provide Promising Targets for Therapy

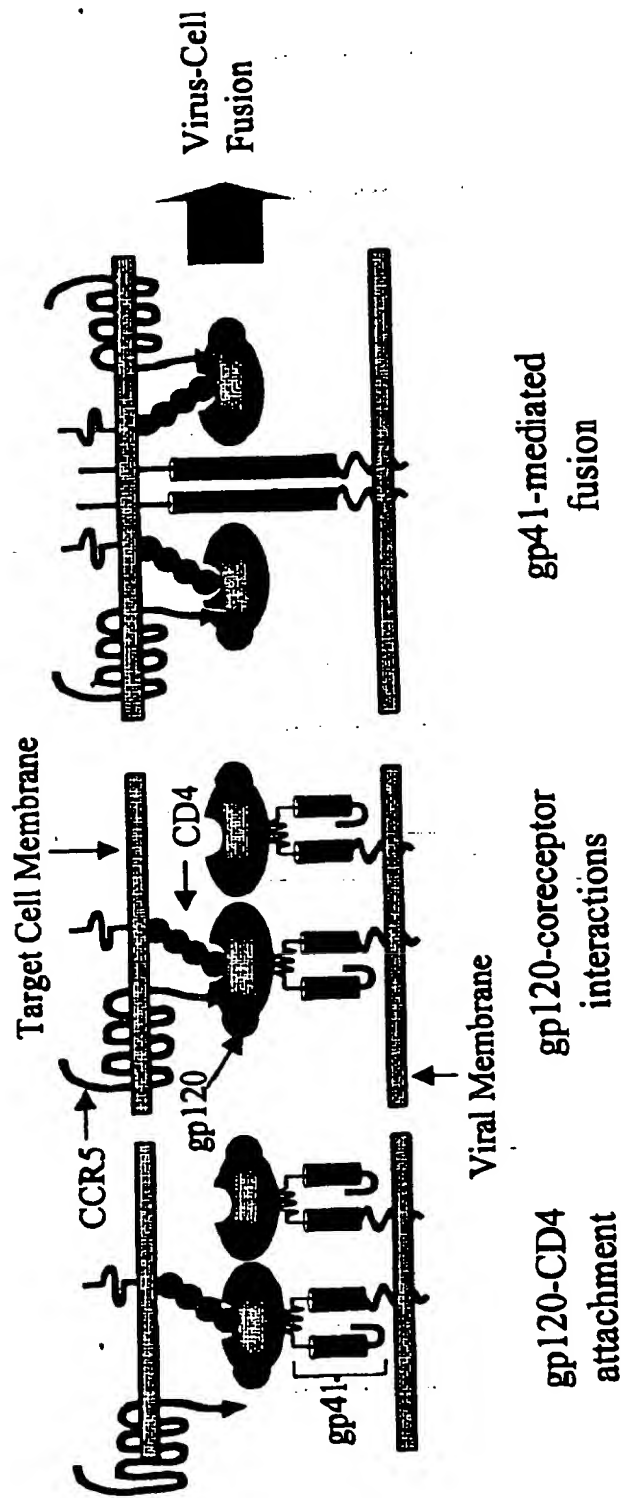


Figure 12

PRO 542 (CD4-IgG2)
attachment inhibitor

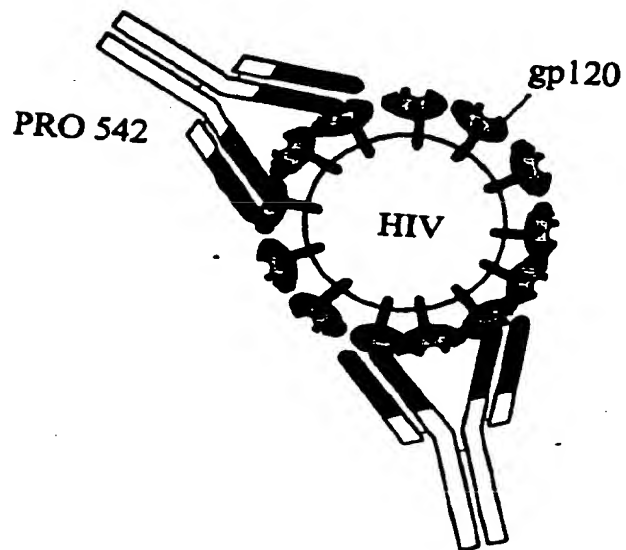


Figure 13

PRO 140
coreceptor inhibitor

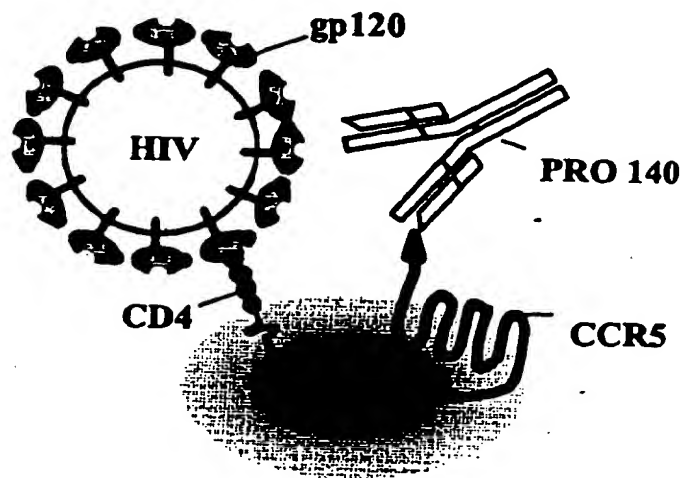
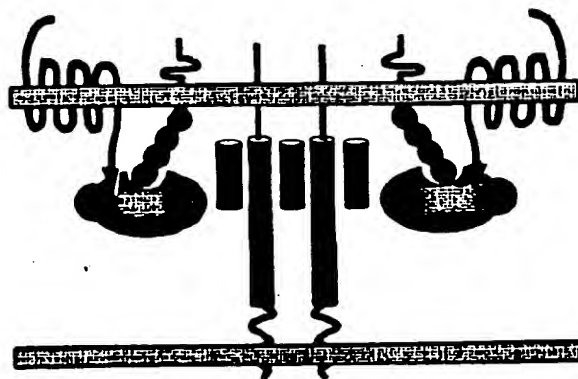


Figure 14

T-20
fusion inhibitor



HIV-1 Virus-Cell Fusion Assay

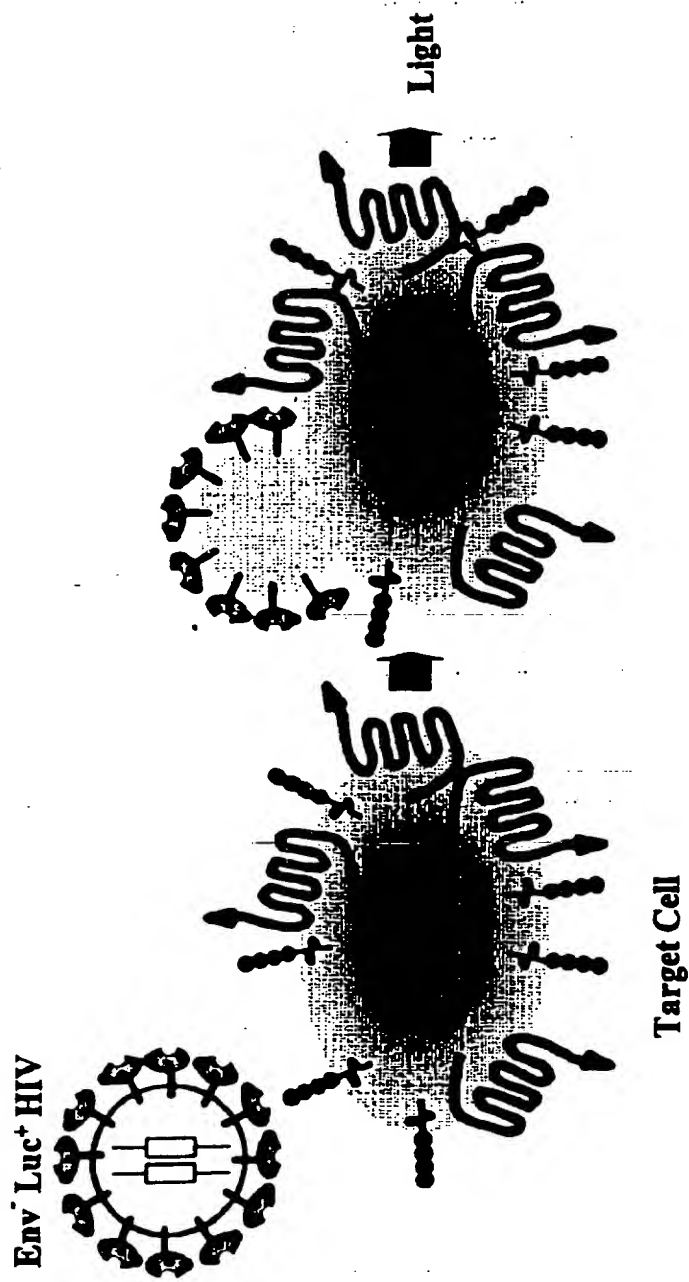
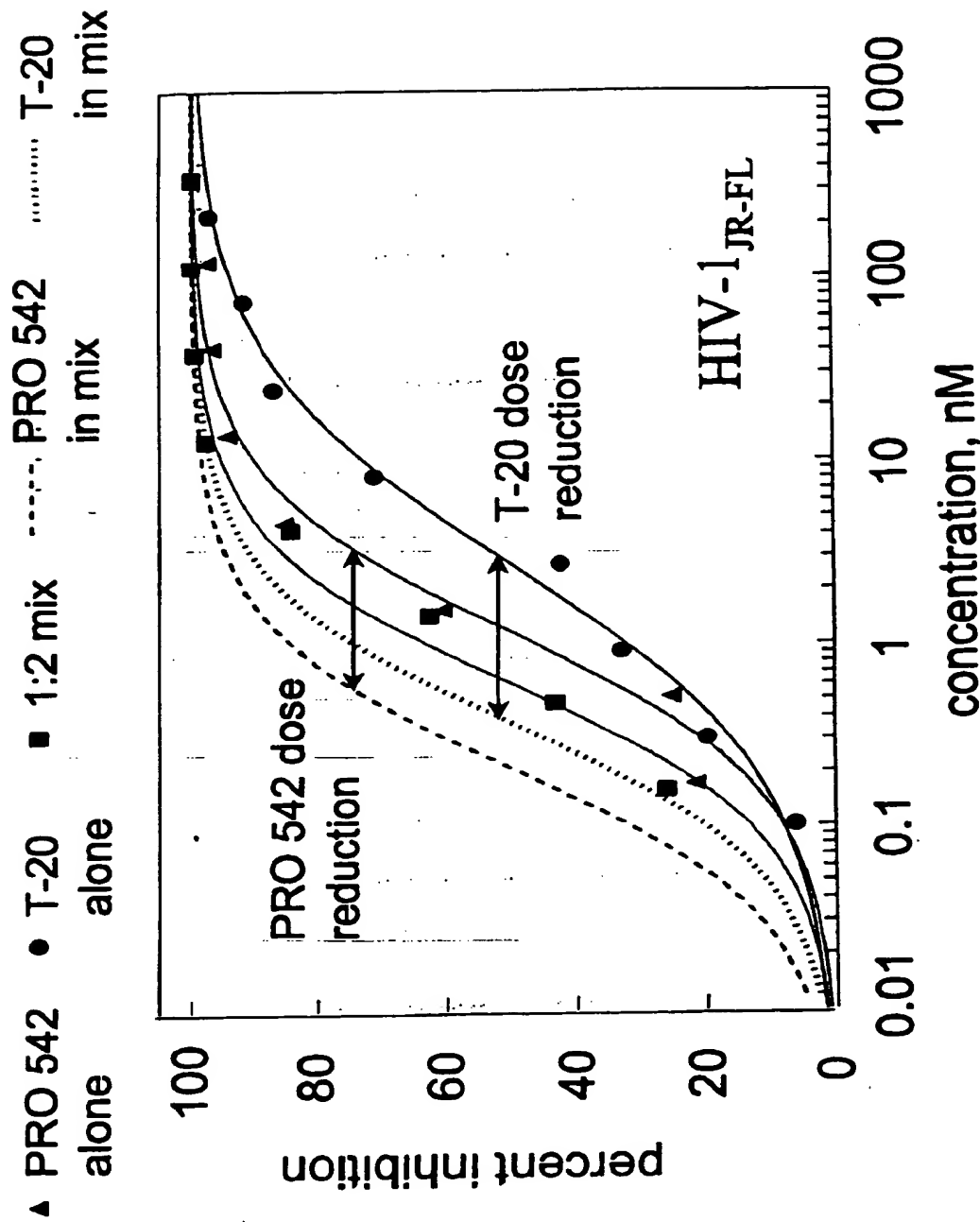


Figure 16

19/32

Synergistic Inhibition of Virus-Cell Fusion with PRO 542 and T-20 (I)



Synergistic Inhibition of HIV-1 Virus-Cell Fusion with PRO 542 and T-20 (II)

Percent Combination		Inhibitory Conc., nM		Dose Reduction	
Inhibition	Index	PRO 542	T-20	PRO 542	T-20
JR-FL 95	0.14	30	120	11	24
(R5) 90	0.18	12	45	8.0	17
70	0.29	2.5	8.0	5.7	10
50	0.39	0.92	2.7	4.4	7.3
DH123 95	0.36	65	123	5.9	6.2
(R5X4) 90	0.45	20	54	4.0	6.1
70	0.76	2.4	12	2.0	5.7
50	1.1	0.64	4.8	1.3	5.5

PRO 542 and T-20 were used in a 1:2 molar ratio

Figure 18

Journal of Cell Biology

HIV-1 Cell-Cell Fusion Assay

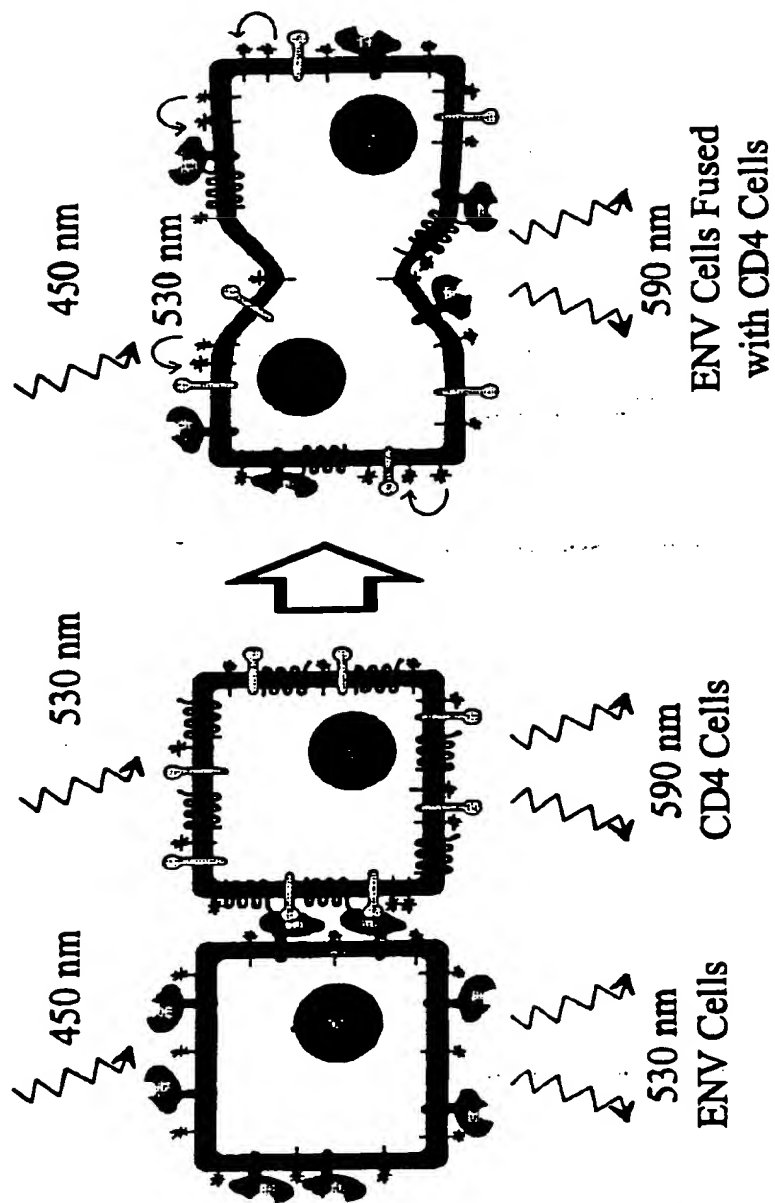


Figure 19

CELL-CELL FUSION

Synergistic Inhibition of Cell-Cell Fusion with PRO 542 and T-20 (I)

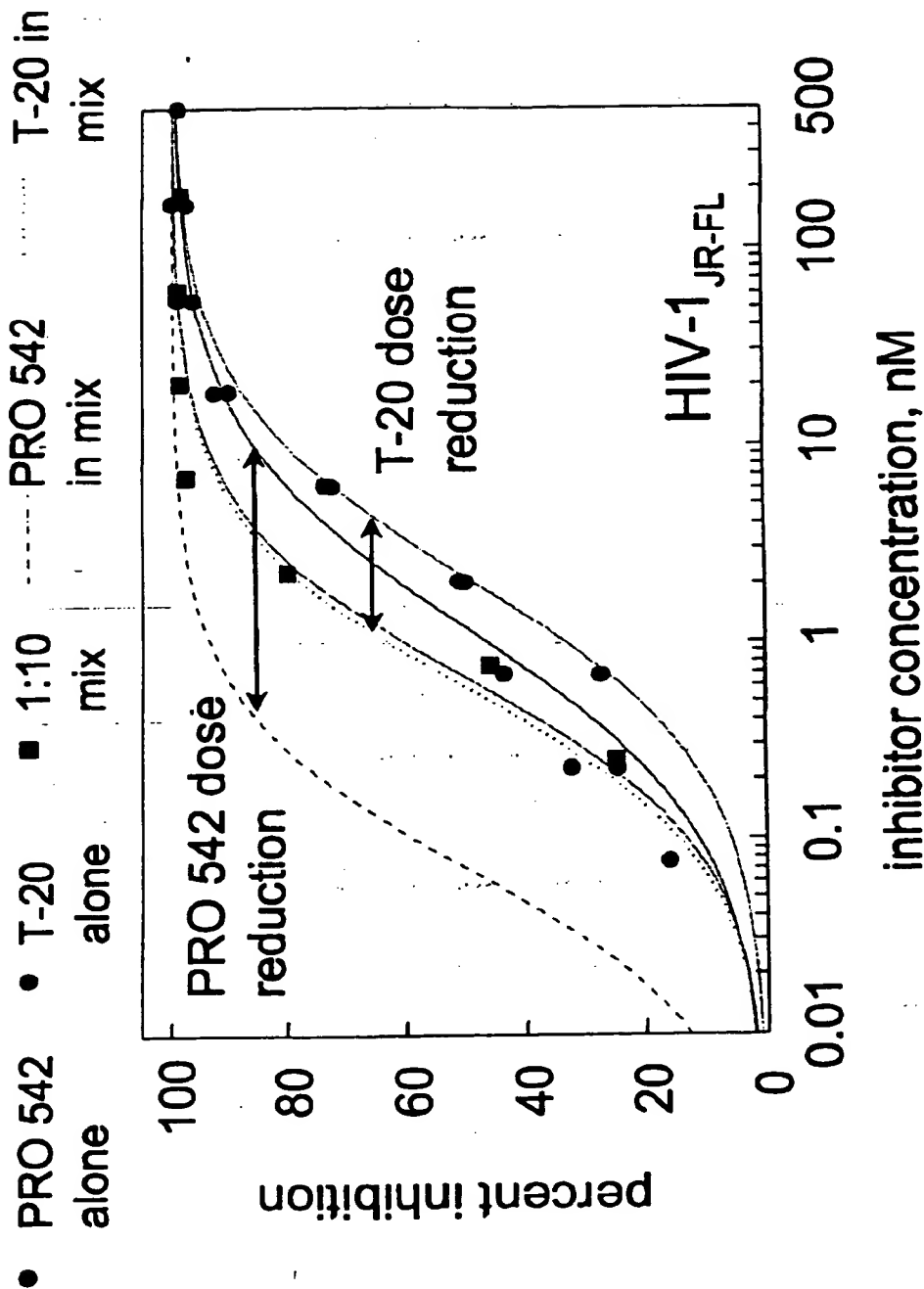


Figure 20

Cell-Cell Fusion

Synergistic Inhibition of HIV-1 Cell-Cell Fusion with PRO 542 and T-20 (II)

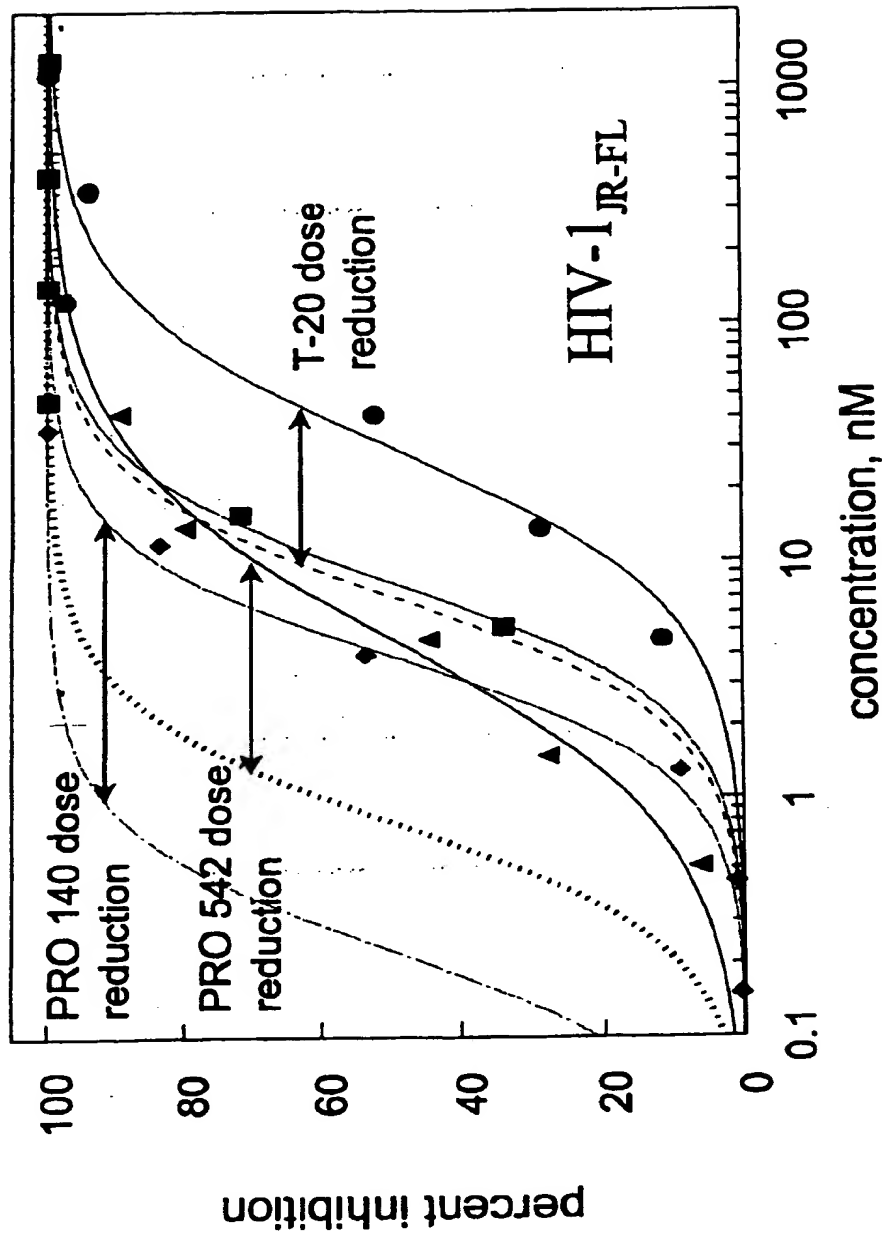
Conc. Ratio	Percent Inhibition	Combination Index	Inhibitory Conc, nM		Dose Reduction (fold)	
			PRO 542	T-20	PRO 542	T-20
1:2	95	0.32	95	47	17	4.9
	90	0.38	39	22	13	4.2
	50	0.69	3.0	2.5	6.2	2.8
1:10	95	0.27	28	58	20	4.8
	90	0.28	11	22	20	4.5
	50	0.34	0.84	1.3	22	3.7
1:50	95	0.33	47	120	56	3.2
	90	0.34	15	42	50	3.2
	50	0.38	0.49	1.8	35	3.0

Virus: HIV-1_{JR-FL}

Figure 21
40550 The 100

PRO 140, PRO 542 and T-20 Triple Combination Synergistically Blocks HIV-1 Entry (I)

- PRO 140 alone ▲ PRO 542 alone • T-20 alone ■ 1:3:30 mix
- PRO 140 in mix PRO 542 in mix - - - - T-20 in mix



PRO 140, PRO 542, T-20 Triple Combination Synergistically Blocks HIV-1 Entry (II)

Percent Inhibition	Combination Index	Inhibitory Conc, nM			Dose Reduction (fold)		
		PRO 140	PRO 542	T-20	PRO 140	PRO 542	T-20
95	0.24	24	61	160	17	12	7.1
90	0.22	23	32	96	21	8.4	7.4
70	0.19	20	9.8	40	32	4.5	8.9
50	0.18	18	4.7	23	41	3.0	10

Inhibition of HIV-1_{JR-FL} mediated cell-cell fusion with PRO 140, PRO 542 and T-20 used in a 1:3:30 molar ratio.

Figure 23
T-20

PRO 542 Does Not Potentiate T-20 Activity in the Absence of Coreceptor

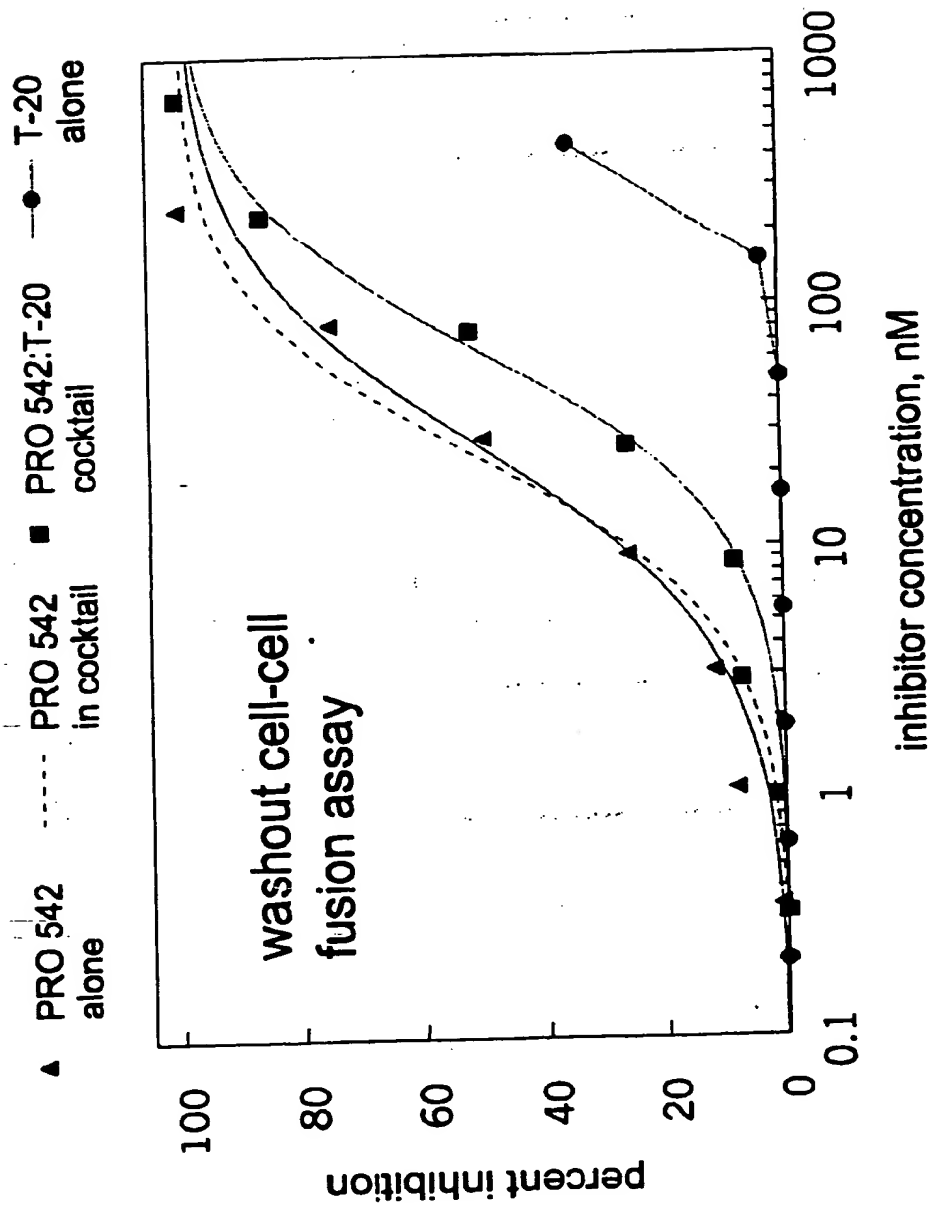


Figure 24

Formation of the Prehairpin Intermediate Requires CD4, Coreceptor and 37 °C (I)

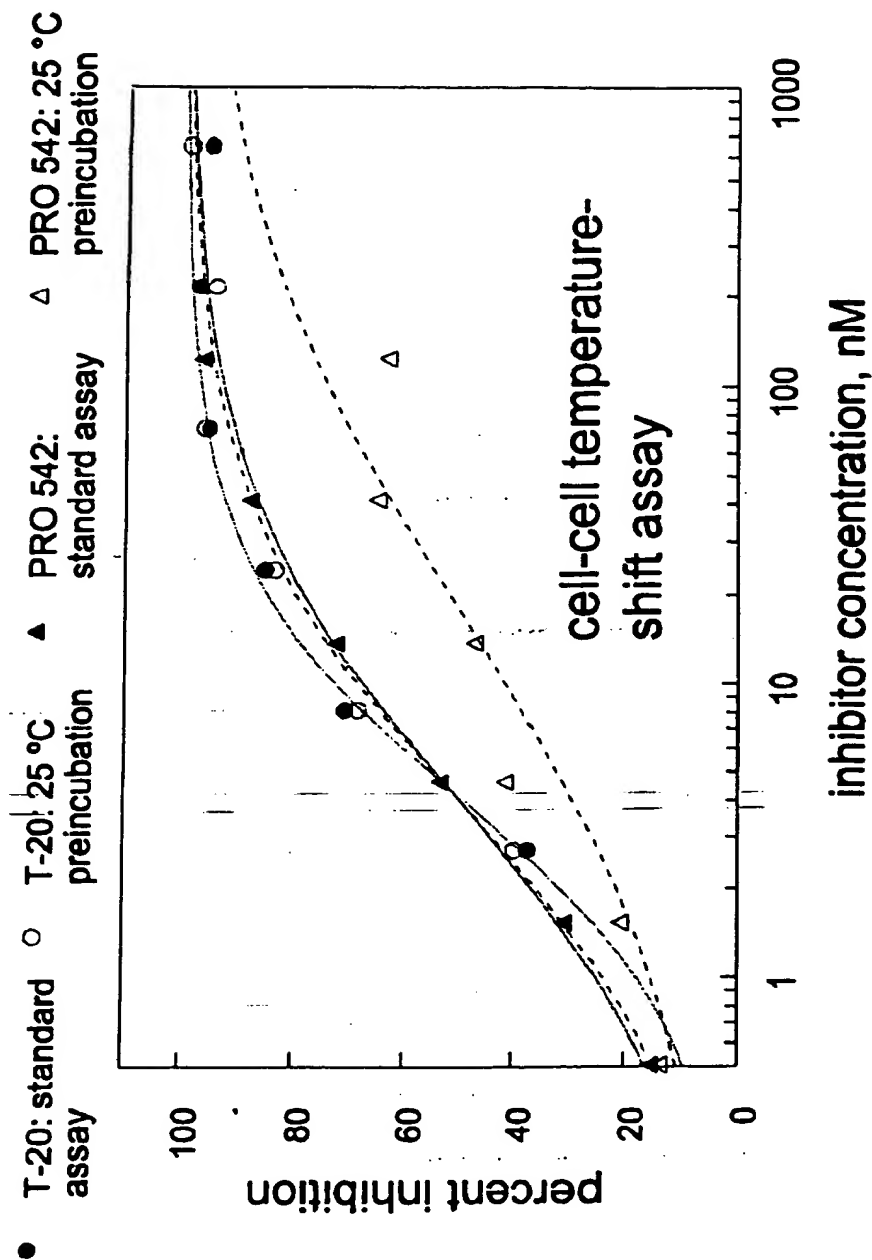


Figure 25

Formation of the Prehairpin Intermediate Requires CD4, Coreceptor and 37 °C (II)

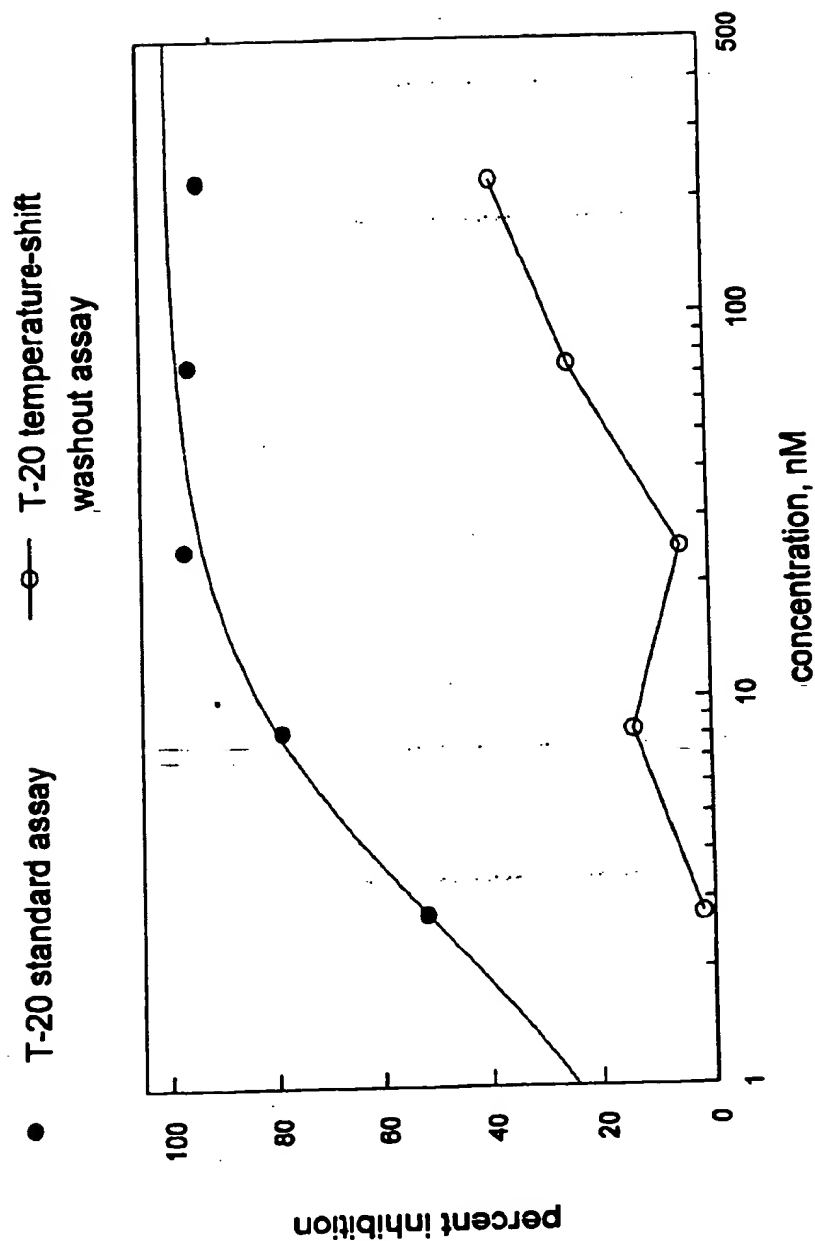


Figure 26

© 1996 by The McGraw-Hill Companies

Possible Mechanism of Synergy. PRO 542 Increases the Half-Life of the T-20 Targets

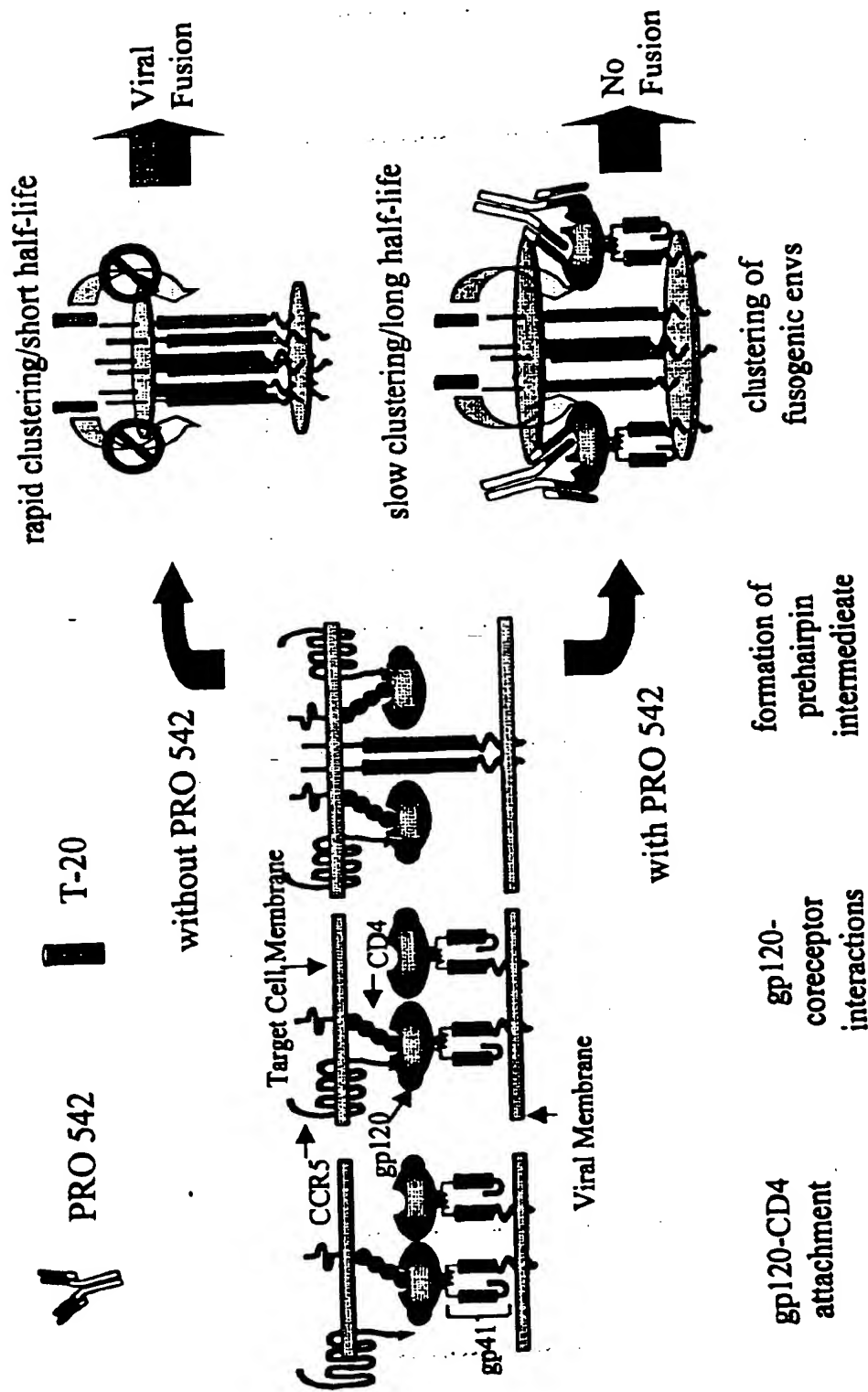


Figure 27 *Figure 27*

Possible Mechanism of Synergy: PRO 542 Increases the Half-Life of the T-20 Targets

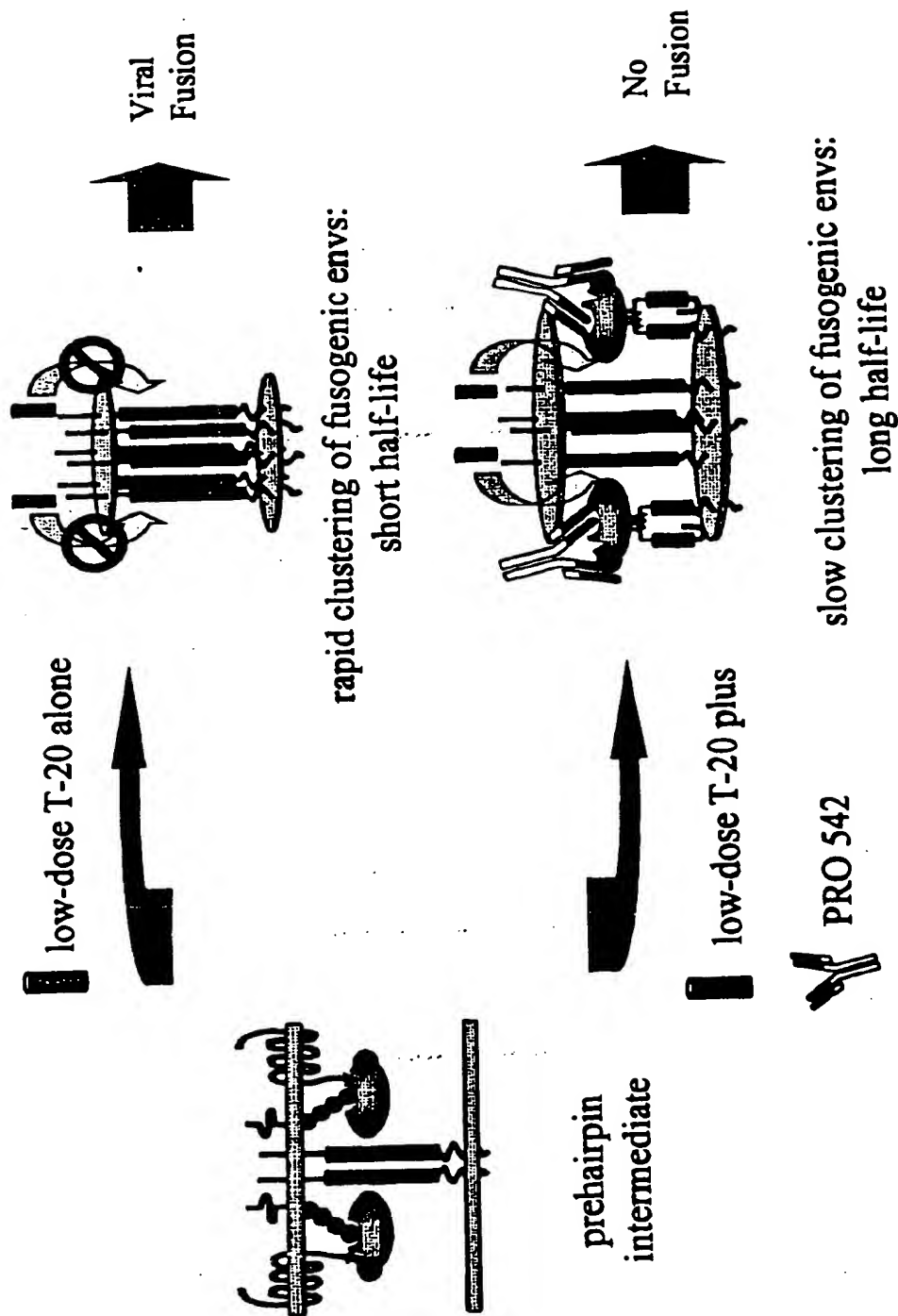


Figure 28 The EC_{50}

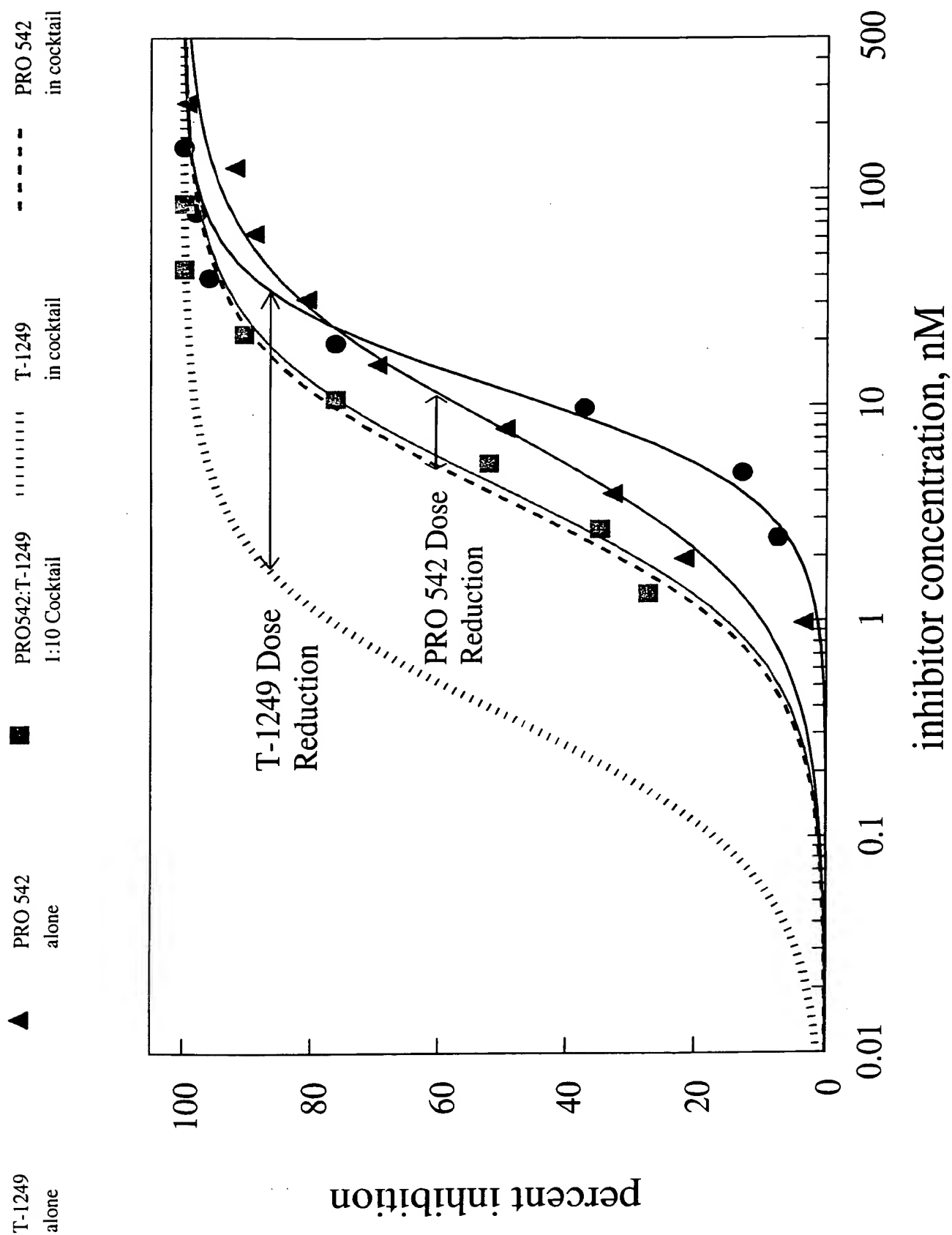


Figure 29

Fraction Inhibited	Dose PRO 542,		Dose T-1249,		Combination Index	Dose	
	nM (alone)	nM (comb)	nM (alone)	nM (comb)		PRO 542	T-1249
0.95	87.90	13.58	37.83	1.36	0.20	6.47	27.86
0.90	48.69	9.52	27.11	0.95	0.24	5.12	28.48
0.85	33.78	7.64	22.06	0.76	0.27	4.42	28.87
0.80	25.65	6.47	18.88	0.65	0.30	3.96	29.17
0.75	20.43	5.65	16.61	0.56	0.32	3.62	29.42
0.70	16.75	5.01	14.85	0.50	0.34	3.34	29.64
0.65	13.99	4.50	13.41	0.45	0.37	3.11	29.84
0.60	11.81	4.06	12.20	0.41	0.39	2.91	30.03
0.55	10.05	3.68	11.13	0.37	0.41	2.73	30.21
0.50	8.57	3.35	10.18	0.33	0.44	2.56	30.39